

# ***In Vitro* Biological Effects of the Antimicrobials Triclocarban, its Analogs, and Triclosan in Enzyme Based Assays**

Ki Chang Ahn<sup>1</sup>, Hsing-Ju Tsai<sup>1</sup>, Christophe Morisseau<sup>1,2</sup>,  
Huazhang Huang<sup>1</sup>, Aman Samra<sup>1</sup>, Daniel P. Y. Chang<sup>3</sup>,  
Shirley J. Gee<sup>1</sup>, Bora Inceoglu<sup>1</sup>  
and Bruce D. Hammock<sup>1,2</sup>

<sup>1</sup>Department of Entomology

<sup>2</sup>U.C.D. Cancer Research Center,

<sup>3</sup>Department of Civil and Environmental Engineering,  
University of California, Davis

Organic residuals symposium, Davis, CA, Oct. 1-2, 2008

# NIEHS-UCD SUPERFUND BASIC RESEARCH PROGRAM HAS DEVELOPED MANY HIGH THROUGHPUT ASSAYS

96 OR 384 WELL  
SCREENS  
ON ENZYMES,  
RECEPTORS,  
AND EVEN  
ORGANISMS



300+ COMPOUNDS  
96 FORMAT DEEP WELL  
ROBOTIC DISPENSING

COMPOUNDS FOR  
ADDITIONAL STUDIES

# ***Antimicrobials Triclocarban and Triclosan in Personal Care Products***



**Triclocarban (TCC) 0.6-1.5%**

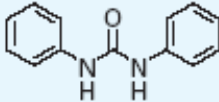
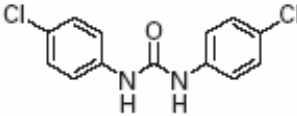
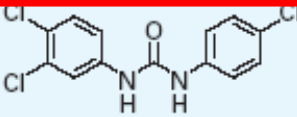
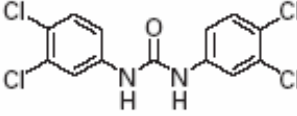
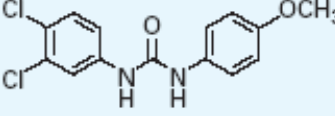
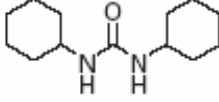
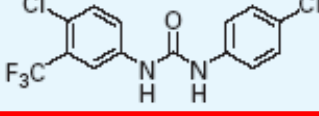
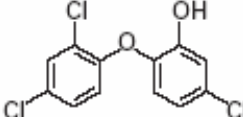


**Triclosan (TCS), 0.3-0.5%**

## ***UCD Research on Antimicrobials TCC and TCS in Personal Care Products***

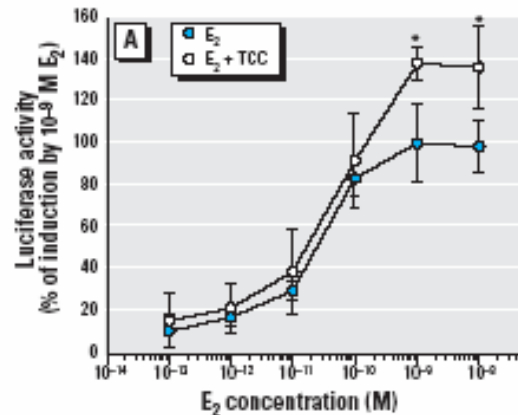
- Dr. Lasley's team: Amplification of transcriptional activity of steroidal hormones by TCC
- Dr. Pessah's team: Sensation of ryanodine receptor involved in calcium signaling by TCS
- Drs. Scow/Young's team: Behavior and remediation of TCC and TCS in the environment
- Drs. Gee/Ahn team: Develop rapid LC-MS and immunoassay methods for trace analysis of TCC and TCS in the environment and human body fluids
- Dr. Hammock's team:
  1. Inhibition of sEH by TCC and carboxylesterase by TCS
  2. Human exposure evaluation

# Chemical Structures of Test Compounds

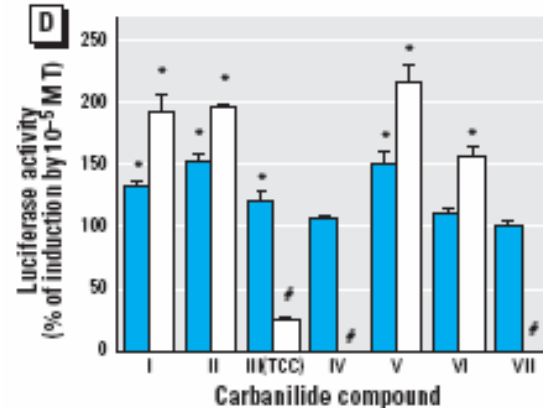
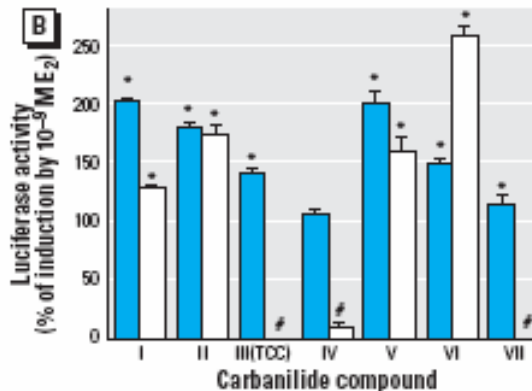
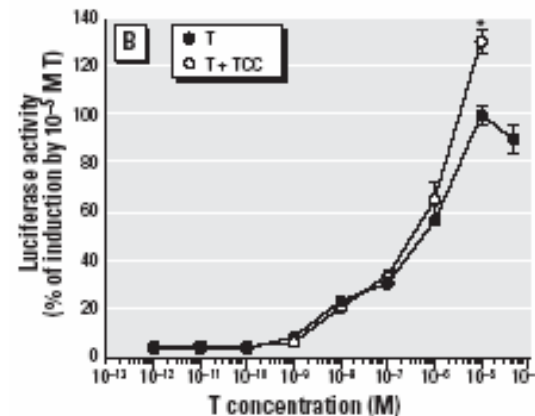
Compound	Common name	Chemical structure	Remark (production)
I	Carbanilide		Possible by-product in the synthesis of the herbicide siduron
II	4,4'-Dichlorocarbanilide		Possible by-product in the synthesis of triclocarban and the insecticide diflubenzuron
III	3,4,4'-Trichlorocarbanilide, triclocarban (TCC)		Antimicrobial agent
IV	3,3',4,4'-Tetrachlorocarbanilide		Possible by-product in the synthesis of triclocarban and the herbicide diuron
V	4'-Methoxy-3,4-dichlorocarbanilide		Structurally related carbanilide compound
VI	1,3-Dicyclohexylurea		By-product in the synthesis of peptide
VII	3-Trifluoromethyl-4,4'-dichlorocarbanilide (TFC)		Antimicrobial agent
VIII	2,4,4'-Trichloro-2'-hydroxydiphenyl ether, triclosan (TCS)		Antimicrobial agent

# ***Androgen-/Estrogen-Based Bioassays:*** ***TCC and analogs amplify the activity of steroid sex*** ***hormones***

## **In ER-based recombinant cells**

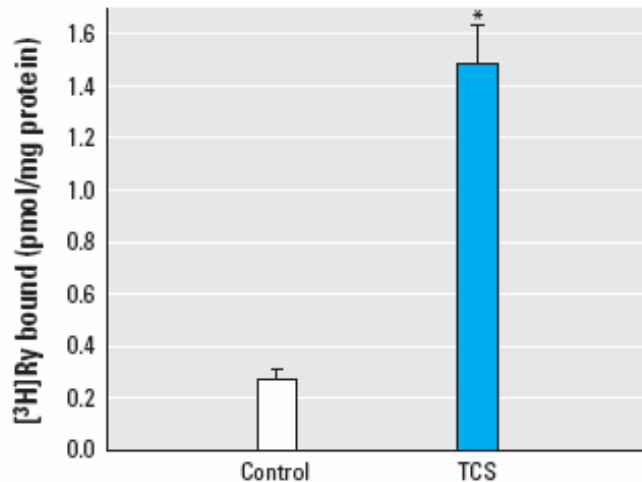


## **In AR-based recombinant cells**



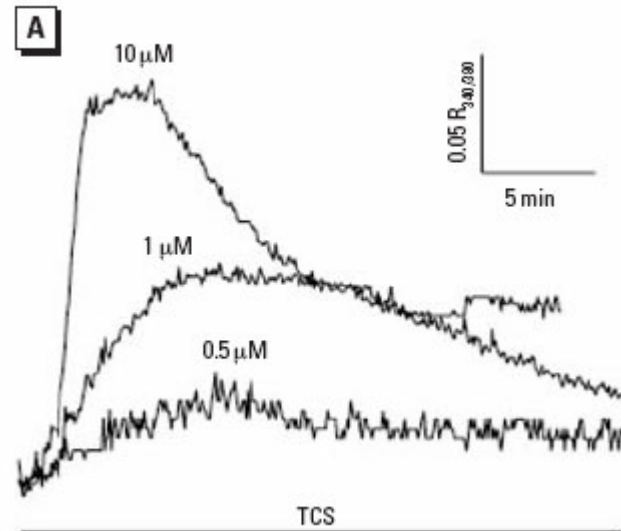
# ***Ryanodine Receptor-Mediated Bioassay:***

**TCS significantly increases [<sup>3</sup>H]ryanodine binding in skeletal muscle SR vesicles.**



**Figure 7.** [<sup>3</sup>H]Ry binding with or without 1.2  $\mu$ M TCS in skeletal muscle sarcoplasmic reticulum vesicles.  
\*Significantly greater than the control at  $p < 0.05$ .

**TCS treatment increases cytosolic Ca<sup>2+</sup> concentration in resting myotubes in a dose-dependent manner.**

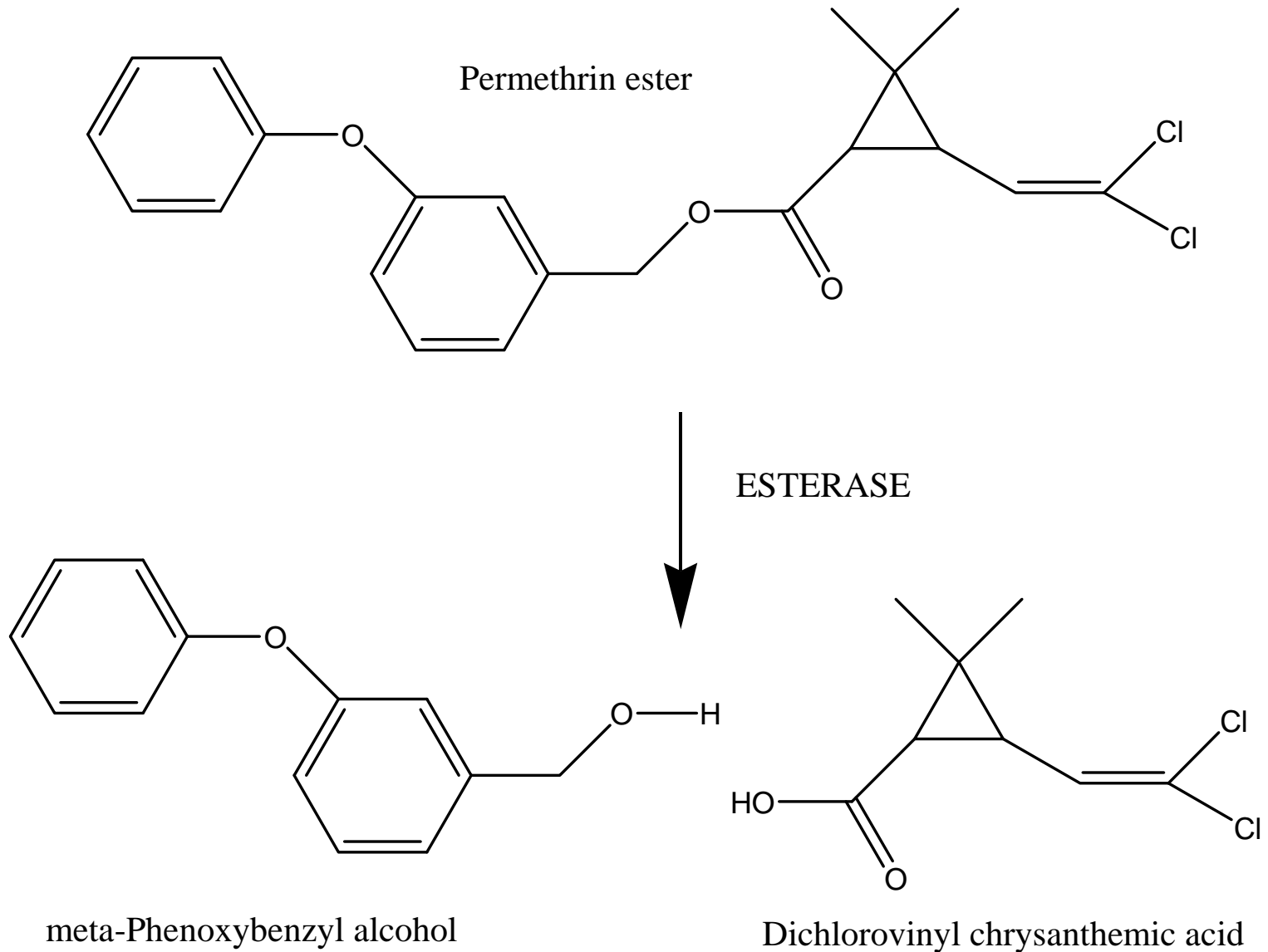


***Enzyme-Based Assays:***  
**TCS inhibits human carboxylesterase 1 (hCE1)**  
**With an IC<sub>50</sub> = 260 nM**

Compound	% Inhibition		
	hCE1	hCE2	hCE3
Benzil	94.7 ± 2.5	77.5 ± 1.3	44.7 ± 1.7
Ethacrynic acid	-	-	-
I	12.3 ± 6.3	22.4 ± 1.3	<1
II	22.0 ± 5.8	10.7 ± 4.7	<1
III (TCC)	13.4 ± 6.0	11.5 ± 3.5	<1
IV	13.4 ± 3.5	8.0 ± 1.3	<1
V	3.0 ± 6.6	11.5 ± 0.8	<1
VI (DCC)	4.0 ± 8.1	6.9 ± 3.7	1.8 ± 2.3
VII (TFC)	24.9 ± 8.8	13.9 ± 6.6	8.2 ± 1.2
VIII (TCS)	83.4 ± 3.5	40.0 ± 5.2	56.2 ± 5.0



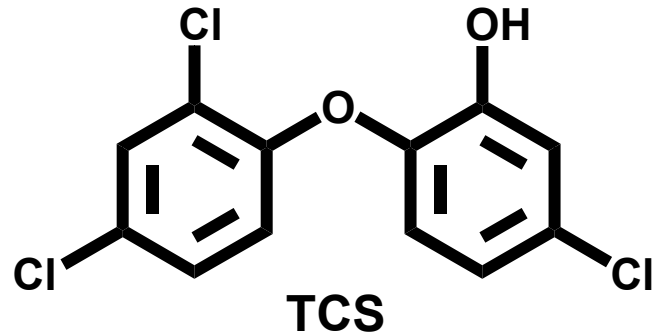
# TCS Inhibits Esterases that Degrade Pesticides and Drugs



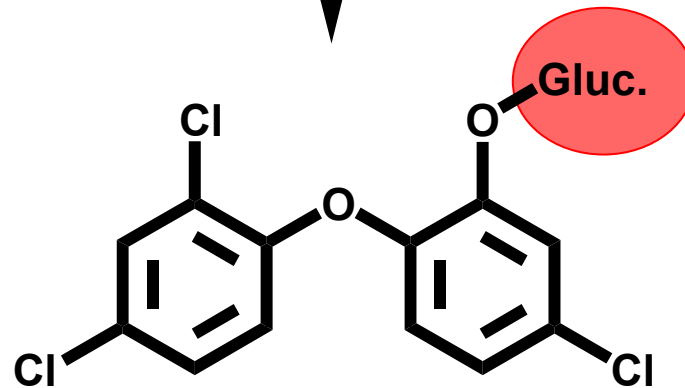
***Enzyme-Based Assays:***  
**Little inhibition of amidase, P450 enzyme (EROD)  
or GST found**

Compound	% Inhibition		Human GSTs
	Microsomal FAAH	EROD	
Benzil	18.2 ± 2.8	-	-
Ethacrynic acid	-	-	52.6 ± 13.3
I	36.8 ± 8.8	25.2 ± 2.6	NI
II	21.9 ± 7.9	3.2 ± 2.5	3.5 ± 0.4
III (TCC)	<1	13.3 ± 3.7	NI
IV	<1	32.1 ± 2.0	NI
V	23.6 ± 4.5	9.8 ± 2.1	NI
VI (DCC)	34.5 ± 3.4	3.3 ± 2.1	NI
VII (TFC)	<1	6.6 ± 3.4	NI
VIII (TCS)	21.7 1.5	12.4 ± 4.5	NI

# ***TCS Metabolism***



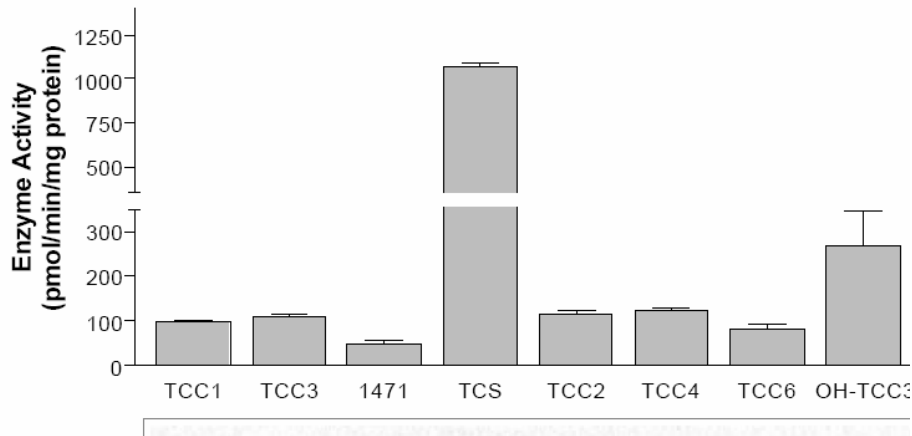
Conjugation



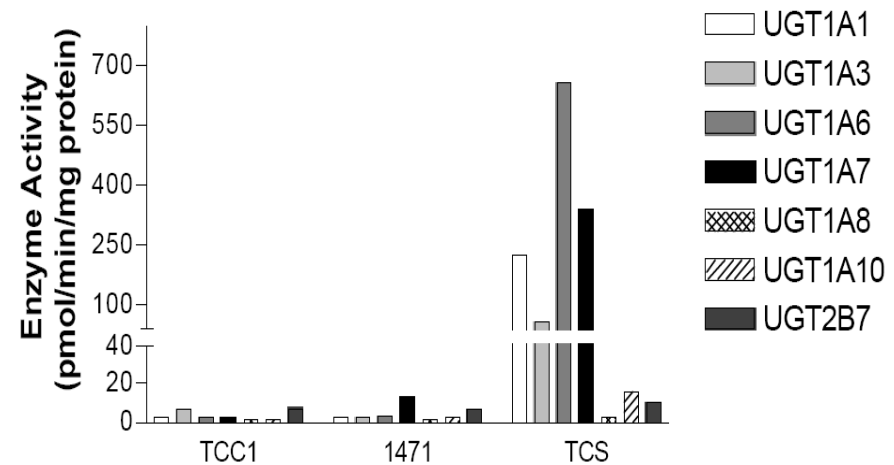
## ***Metabolism to Glucuronide Conjugates:***

- TCC and its analogs form direct *N*- or *N'*-glucuronides in both human and murine liver microsomes slowly
  - TCS forms an *O*-glucuronide at a high rate driven by UGT1A6 and other isoforms
- TCS could competitively inhibit other key glucuronidation**

Mouse Liver Microsome  
UGT Assay



COS cells (transfections)

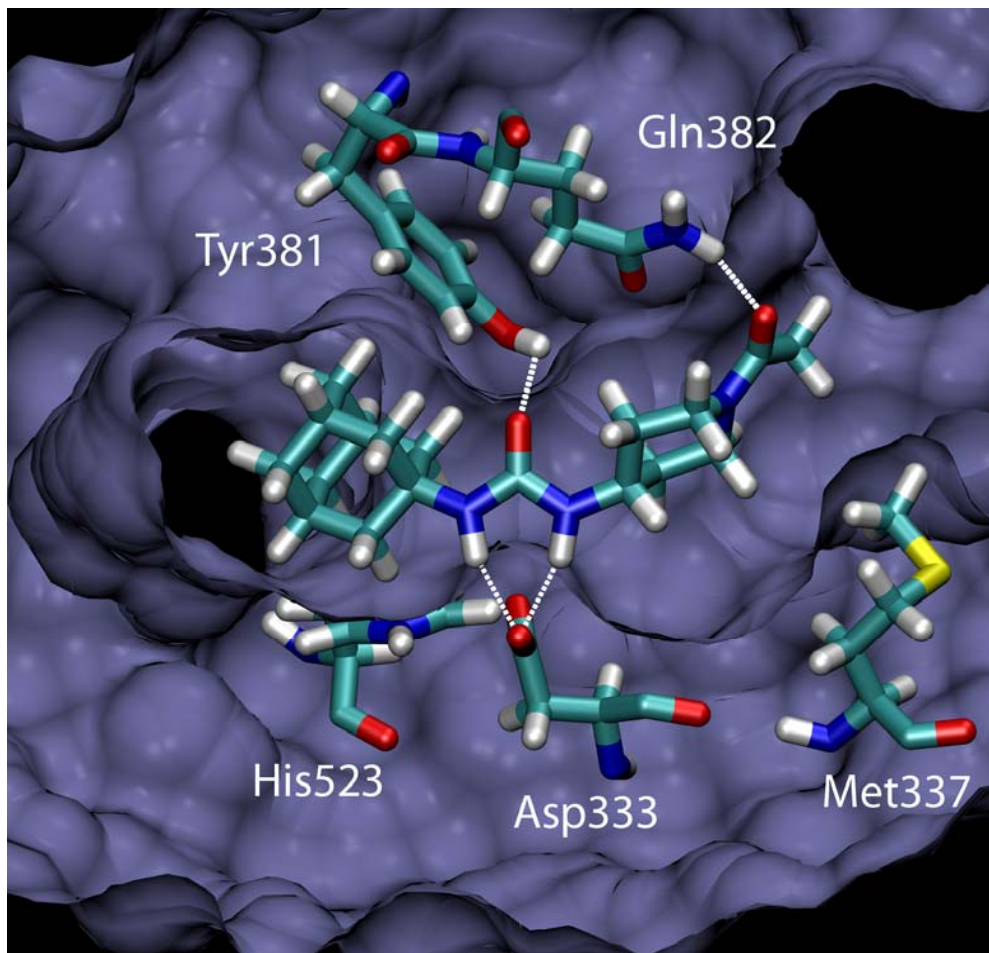


# ***Enzyme-Based Assays:***

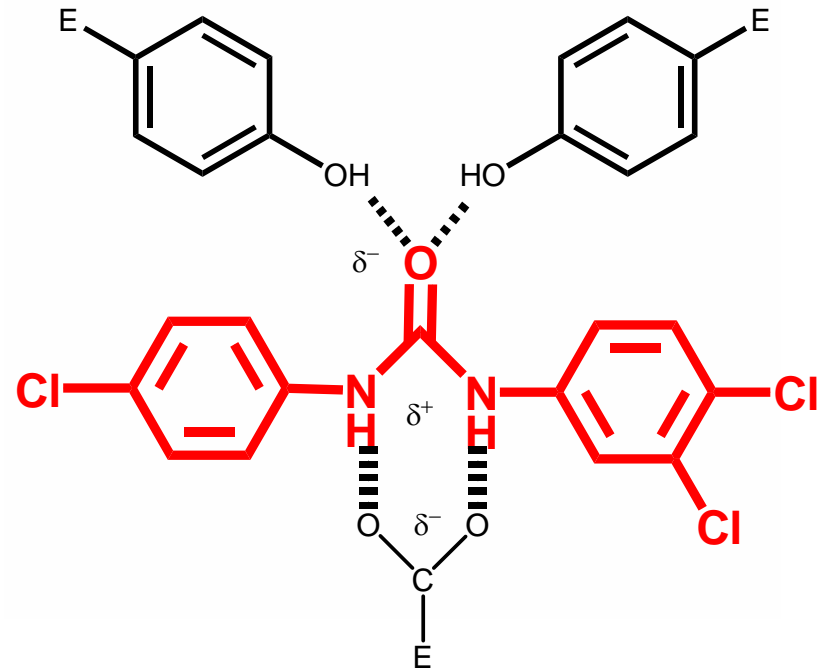
## **TCC and its analogs strongly inhibit sEH**

Compound	IC <sub>50</sub> (nM)	
	mEH	sEH
AUDA	>100000	3 ± 0.02
NSPA	390 ± 10	-
I	>100000	390 ± 3
II	>100000	59 ± 4
III (TCC)	>100000	13 ± 1
2'-OH TCC	>100000	73 ± 5
2'-OH TCC (sulfate conjugate)	>100000	101 ± 4
IV	>100000	28 ± 2
V	>100000	18 ± 1
VI (DCC)	> 20000	52 ± 3
VII (TFC)	> 10000	16 ± 1
VIII (TCS)	>100000	4764 ± 123

A

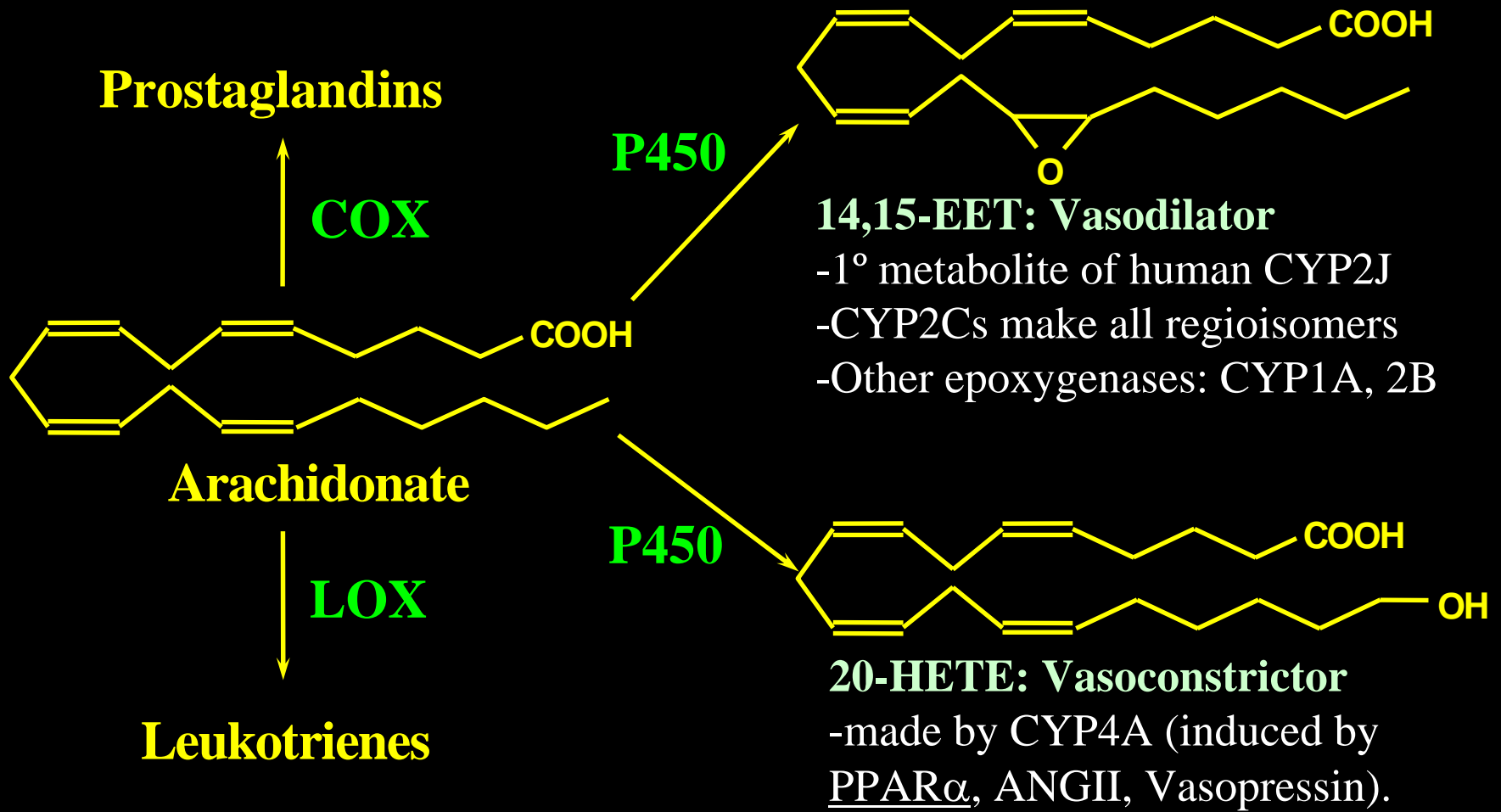


B



**The central pharmacophore binds as a partial salt bridge stabilized by two H bonds. The tyrosines polarize the carbonyl. There are hydrophobic pockets on either side of the catalytic site with few polar residues.**

# Arachidonic Acid Metabolism



DHETs

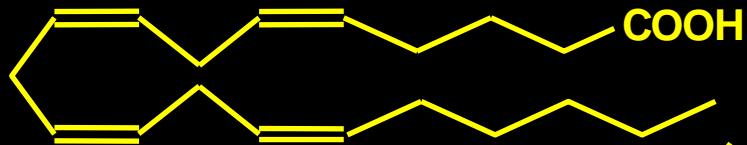
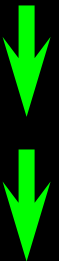
sEH

EETs

sEH inhibitors or  
sEHI  
target sEH

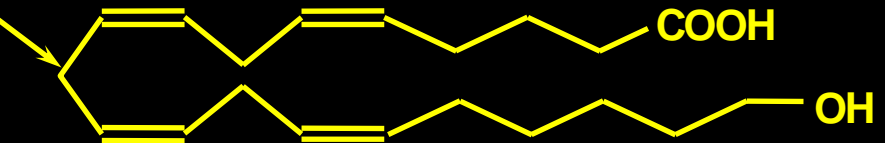
P450

HYPERTENSION  
INFLAMMATION

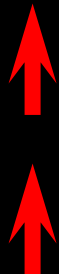


Arachidonate

P450

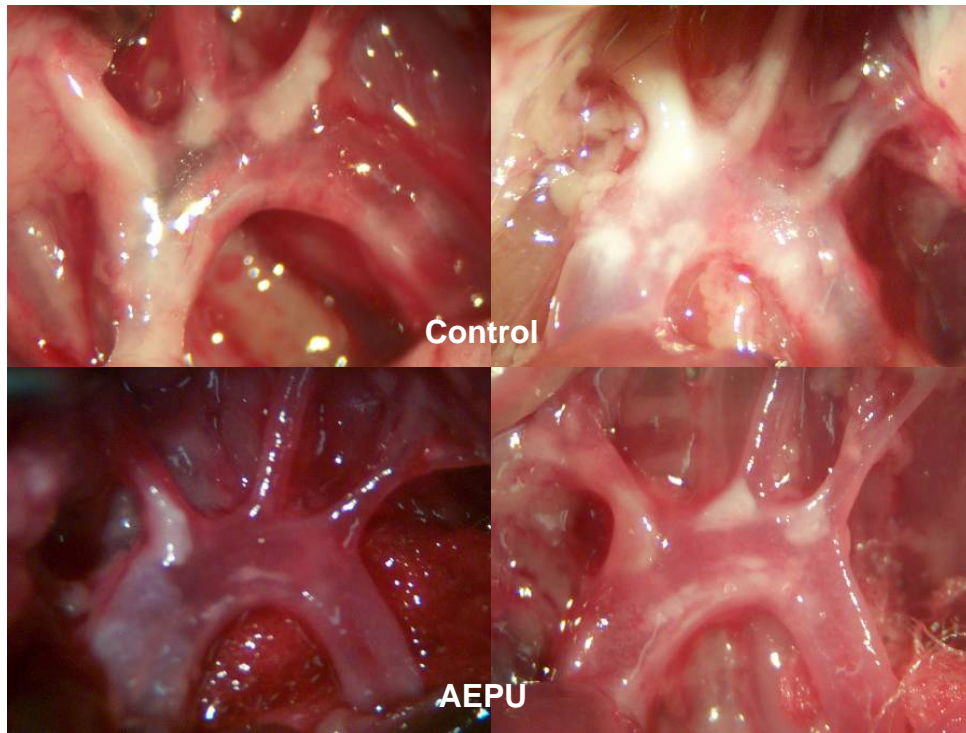


HYPERTENSION  
INFLAMMATION

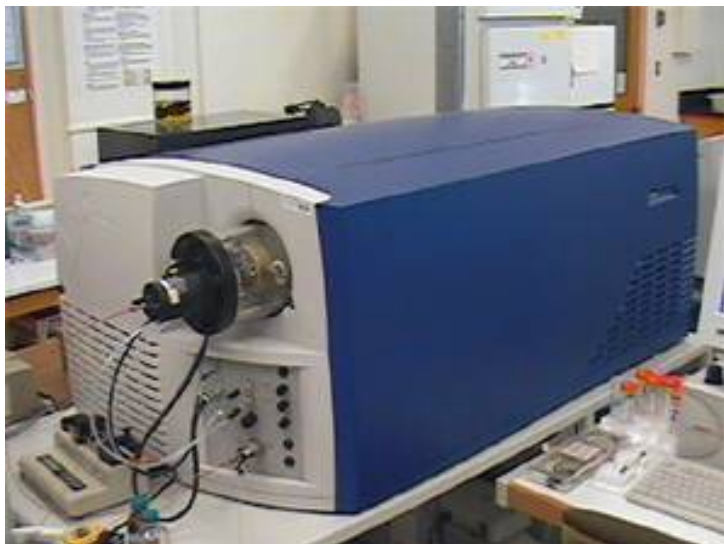




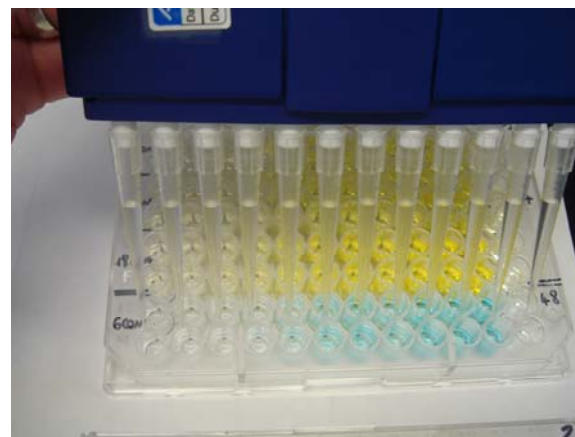
# sEHI BLOCK HARDENING OF THE ARTERIES (LEFT) AND HEART FAILURE (RIGHT)



# ***Analytical Methods using a Small Volume ( $\mu$ L) of Sample Allow Monitoring of TCC & TCS***

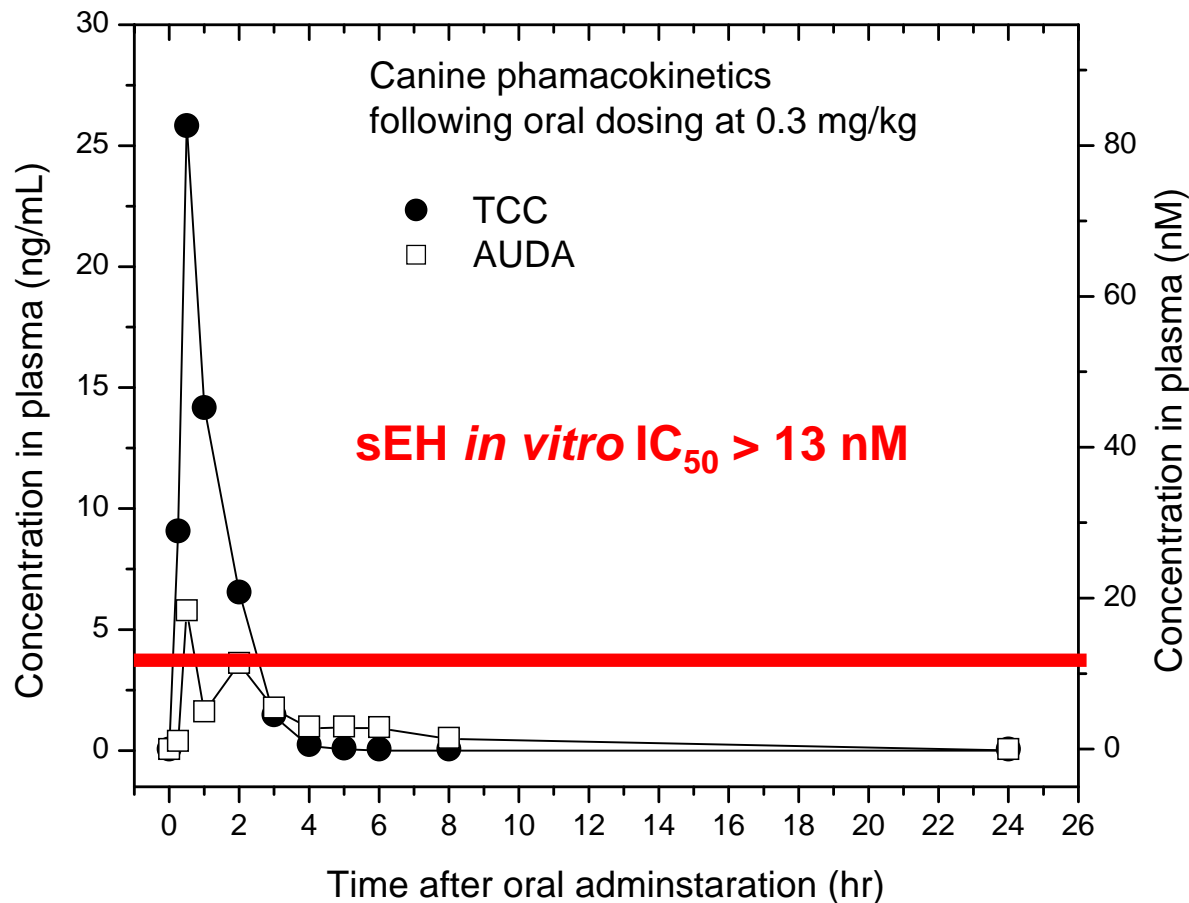


- **Instrumental LC/MS/MS analysis**
  - Sensitive, - Selective
  - Precise
- **Immunological analysis**
  - Cost effective
  - Rapid, - Sensitive - Biosensor



# ***Canine Exposure to TCC:***

## **TCC concentrations in blood over time after oral administration of 0.3 mg/kg body weight**



# CONCLUSIONS

- **TCS inhibits human carboxylesterase that is responsible for hydrolysis of many xenobiotics.**
- **TCS is rapidly degraded by glucuronidation, but could be a competitive inhibitor of other xenobiotics in this pathway.**
- **TCC and its analogs, strongly inhibit sEH *in vitro*.**
- **sEH inhibitors stabilize endogenous epoxy lipids, some of which are chemical mediators.**
- **They reduce high blood pressure, are strongly anti-inflammatory, synergize NSAIDs and aspirin, and have analgesic properties. The high potency of TCC on sEH suggests that it could be therapeutically effective with dermal application. TCC is more active than drugs for this target in clinical trials.**

# ACKNOWLEDGEMENTS

- Laboratory of Environmental Toxicology, Departments of Chemistry & Biochemistry and of Pharmacology, University of California San Diego, La Jolla, California,
  - Dr. Robert H. Tukey
  - Dr. Mei-Fei Yueh
- NIEHS Superfund Basic Research Program (5P42 ES04699);
- NIEHS (R37 ES02710);
- UC Davis Center for Children's Environmental Health and Disease Prevention (1PO1 ES11269)
- NIOSH Center for Agricultural Disease and Research, Education and Prevention (1U50 OH07550).



## ***Antimicrobial TCC and TCS as High Production Volume Personal Care Chemicals***

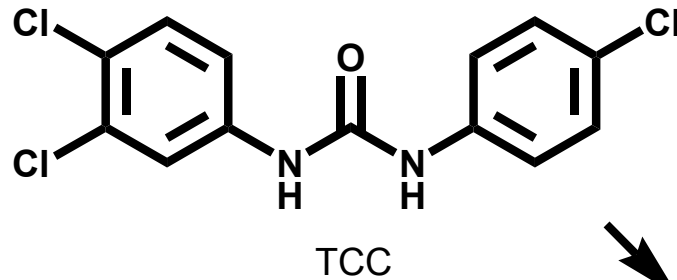
	Personal care products	Content	U.S.A. annual production (Estimated)	U.S.A. market (Estimated)
Triclosan (TCS)	Liquid soap, oral care products, Plastic kitchen wares, plastic toys,	Up to 1%	One million pounds	One billion dollars
Triclocarban (TCC)	Solid bar soap, deodorants	Up to 1.2%	One million pounds	100 million dollars

# ***Enzyme-Based Assays***

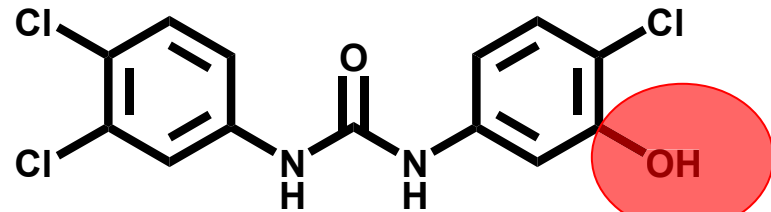
- *As an approach to evaluation of safety, we tested effects of TCC and TCS on several in vitro enzyme systems.*
- **OBJECTIVES:** We tested TCC, its analogs, and TCS as inhibitors of the recombinant human microsomal fatty acid amide hydrolase (FAAH), three recombinant human carboxyl esterases, human microsomal EROD activity (largely P450 1A1), human soluble glutathione transferase activity, and human recombinant soluble and microsomal epoxide hydrolase activity (mEH and sEH), and UDP-glucuronosyltransferase (UGT) activity.
- **METHODS:** A novel fluorescent assay was used for FAAH, cyanohydrin based fluorescent assays were used for esterases and sEH, EROD for P450, chlorodinitrobenzene for GST, and *cis*-stilbene oxide for mEH.



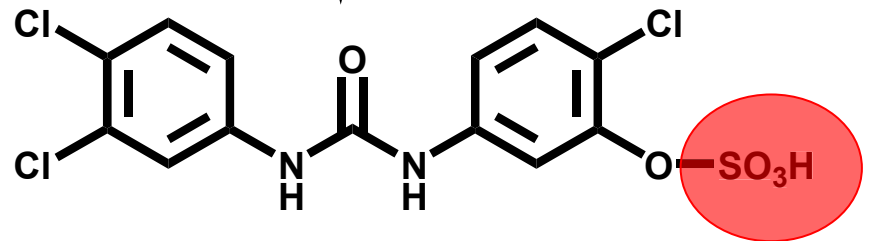
# ***TCC Metabolism***



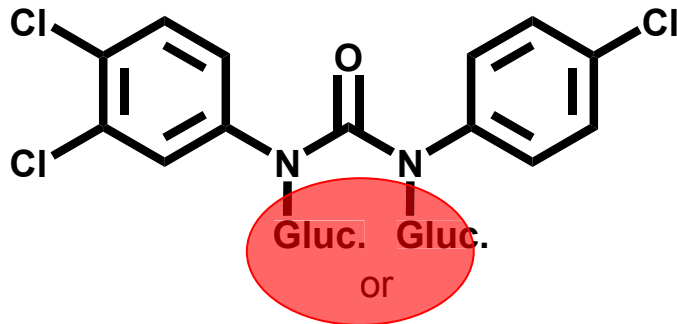
Hydroxylation



Conjugation



Conjugation



**Structure of TCC in bold shown interacting with a nucleophilic aspartic acid and two polarizing tyrosines in the sEH enzyme (E).**

